

TRS

TOXICOLOGY/REGULATORY SERVICES, INC.

201-15016

December 31, 2003

Mr. Mike Leavitt, Administrator
US Environmental Protection Agency
PO Box 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know Program, AR-201

Re: Test Plans for CAS RNs 54395-52-7 and 38103-06-9

Toxicology/Regulatory Services (TRS) is submitting two Test Plans/Robust Summaries on behalf of General Electric Company – Plastics (GE Plastics; Registration Number 1100342). Please add the attached Test Plans and Robust Summaries for 1H-Isoindole-1,3(2H)-dione, 5,5'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis[2-methyl- (Bisphenol A Bisimide; CAS RN 54395-52-7) and 1,3-Isobenzofurandione, 5,5'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis- (Bisphenol A Dianhydride; CAS RN 38103-06-9) sponsored by GE Plastics to the list of those chemicals to begin testing in 2003. Please address any comments to:

Dr. Ronald Joiner
Manager, Global Toxicology
General Electric Company
One Plastics Avenue
Pittsfield, MA 01201
Phone: 413-448-6323; Fax: 866-607-2387
EMAIL: Ronald.Joiner@GEP.GE.COM

Thank you,

John P. Van Miller

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U.S. HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

ROBUST SUMMARY

1H-Isoindole-1,3(2H)-dione, 5,5'-[(1-methylethylidene)
bis(4,1-phenyleneoxy)]bis[2-methyl-
(Bisphenol A Bisimide; CAS RN 54395-52-7)

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Prepared by:
General Electric Company
Pittsfield, MA, USA

Prepared for:
U.S. Environmental Protection Agency
Washington, D.C., USA

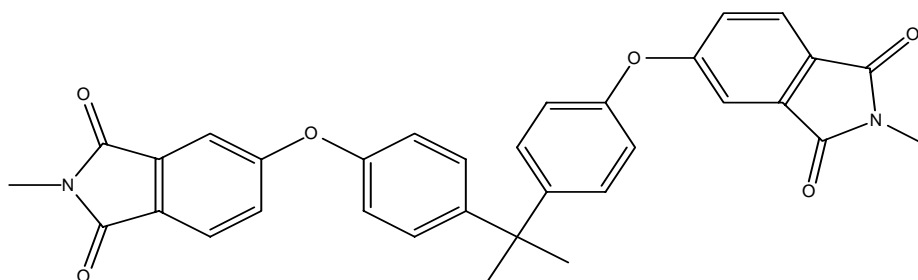
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CHEMICAL IDENTITY AND USE INFORMATION**CAS RN**

54395-52-7

CHEMICAL NAME

1H-Isoindole-1,3(2H)-dione, 5,5'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis[2-methyl- (hereafter called, Bisphenol A Bisimide)]

STRUCTURE, MOLECULAR FORMULA, MOLECULAR WEIGHTMolecular Formula: $C_{33}H_{26}N_2O_6$

Molecular Wt.: 546.57

OTHER CHEMICAL IDENTITY INFORMATION

4,4'-((Isopropylidene)bis(p-phenyleneoxy))bis(N-methylphthalimide-)

Bisphenol A Bisimide

Bisphenol A Diimide

N,N'-Dimethyl-2,2-bis(4-(3,4-dicarboxyphenoxy)phenyl)propane diimide

BI

BI (BPA-Bisimide)

BPA-BI

BPA-Bisimide

UI80-3

PURITY

Typical purity of 4,4'-BPA-BI is 94 wt%. Two isomers, 3,3'- and 3,4'-BPA-BI comprise approximately 6 wt% yielding a purity of all isomers > 99.9 wt%.

USE PATTERN

BPA-BI is a chemical intermediate that is primarily used as a reactive intermediate to make high molecular weight polyetherimide polymers.

TEST PLAN

Bisphenol A Bisimide CAS RN: 54395-52-7		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	Testing Required
STUDY		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
PHYSICAL AND CHEMICAL DATA								
1.0	Melting Point	N	N	N	N	N	N	Y
2.0	Boiling Point	N	N	N	N	N	N	Y
3.0	Vapor Pressure	N	N	N	N	N	N	Y
4.0	Partition Coefficient	N	N	N	N	N	N	Y
5.0	Water Solubility	N	N	N	N	N	N	Y
ENVIRONMENTAL FATE AND PATHWAY								
6.0	Photodegradation	N	N	N	N	N	N	Y
7.0	Stability in Water	N	N	N	N	N	N	Y
8.0	Transport and Distribution	N	N	N	N	N	N	Y
9.0	Biodegradation	N	N	N	N	N	N	Y
ECOTOXICITY								
10.0	Acute Toxicity to Fish	N	N	N	N	N	N	Y
11.0	Toxicity to Algae	N	N	N	N	N	N	Y
12.0	Acute Toxicity to Daphnia	N	N	N	N	N	N	Y
TOXICITY								
13.0	Acute Toxicity	Y	N	N	Y	N	Y	N
14.0	Genotoxicity <i>In Vitro</i> or <i>In Vivo</i> (Chromosome Aberration Tests)	Y	Y	Y	N	N	Y	N
15.1	Genotoxicity <i>In Vitro</i> (Bacterial Test)	Y	N	N	Y	N	Y	N
15.2	Genotoxicity <i>In Vitro</i> (Mammalian Cells)	Y	Y	Y	N	N	Y	N
16.0	Repeated Dose Toxicity	Y	N	Y	N	N	Y	N
17.0	Reproductive Toxicity	N	N	N	N	N	N	Y
18.0	Developmental Toxicity / Teratogenicity	Y	N	Y	Y	N	Y	N

ROBUST SUMMARY**201-15016B****PHYSICAL AND CHEMICAL DATA****1.0 MELTING POINT**

No data were found. Study according to OECD Guideline 102 in progress.

2.0 BOILING POINT

No data were found. Study according to OECD Guideline 103 in progress.

3.0 VAPOR PRESSURE

No data were found. The calculated vapor pressure of BPA-BI was less than the detection limit of the most sensitive (gas saturation) method. Therefore, in lieu of conducting a study according to OECD Guideline 104, an Expert Statement will be prepared describing the scientific rationale for not determining the vapor pressure.

4.0 PARTITION COEFFICIENT ($\text{Log}_{10}P_{ow}$)

No data were found. Study according to OECD Guideline 107 in progress.

5.0 WATER SOLUBILITY**5.1 SOLUBILITY**

No data were found. Study according to OECD Guideline 105 in progress.

5.2 pH VALUE, pKa VALUE

No studies were found.

ENVIRONMENTAL FATE AND PATHWAYS**6.0 PHOTODEGRADATION**

When physical/chemical properties testing is complete, the resulting data will be coupled with modeling efforts to predict the environmental fate and pathways of Bisphenol A Bisimide.

7.0 STABILITY IN WATER

No data were found. Study according to OECD Guideline 111 in progress.

8.0 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS, INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS**8.1 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

When physical/chemical properties testing is complete, the resulting data will be coupled with modeling efforts to predict the environmental fate and pathways of Bisphenol A Bisimide.

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from International Research and Development Corporation, Mattawan, MI, USA.
 Reliability: (Klimisch Code 2) Valid with restrictions. Only four animals dosed, two per group.

13.2 ACUTE INHALATION TOXICITY

No studies were found.

13.3 ACUTE DERMAL TOXICITY

Type: LD₀ [] LD₁₀₀ [] LD₅₀ [] LD_{L0} [] Other [X]
 Species/Strain: Rabbit/New Zealand White
 Value: Not applicable
 Method: Not specified
 GLP: Yes [] No [X] ? []
 Test Substance: AR No. 82895 (Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7); from General Electric Company; Purity: See “Chemical Identity and Use Information” section.
 Remarks: Two New Zealand White rabbits (one male and one female) were used at each of two doses. The rabbits weighed from 2432 to 2747 grams at study initiation. Body weights were measured initially and at 14 days. The compound was applied to the clipped back of each rabbit. Two rabbits received 200 mg of the test substance/kg body weight and two rabbits received 2000 mg of the test substance/kg body weight. The application area was wrapped with a gauze bandage and occluded with plastic wrap. After 24 hours, the bandages were removed and the application areas were washed with tepid tap water. The rabbits were observed for mortality for a period of 14 days.
 Results: All rabbits survived the 14 day observation period. Three of the rabbits exhibited body weight gains and one rabbit showed a slight (151 gram) loss in body weight during the 14-day observation period.
 Reference: Wazeter, F. X. and E. I. Goldenthal (1974) Unpublished report for Project No. 313-034 entitled “Acute Toxicity Screening Studies in Rats and Rabbits” dated May 16, 1974 for General Electric Company, from International Research and Development Corporation, Mattawan, MI, USA.
 Reliability: (Klimisch Code 2) Valid with restrictions. Only two rabbits exposed per group.

14.0 GENETIC TOXICITY *IN VITRO* OR *IN VIVO* (CHROMOSOMAL ABERRATIONS)

Type: *In vitro* mammalian chromosome aberration test
 System of testing: Chinese hamster ovary (CHO) cells
 Concentration: 0, 6.25, 12.5, 25, 35, 50, 75, 100 µg/mL (4-hr treatment w/o S9 mix)
 0, 6.25, 12.5, 25, 35, 50, 75, 100, 125 µg/mL (20-hr treatment w/o S9 mix)
 0, 6.25, 12.5, 25, 50, 75 µg/mL (4-hr treatment with S9 mix)
 Metabolic activation: With []; Without []; With and Without [X]; No data []
 Results: Negative with and without metabolic activation
 Cytotoxicity conc: With metabolic activation: None
 Without metabolic activation: ≥ 450 µg/mL (4 hr exposure)
 Without metabolic activation: ≥ 150 µg/mL (20 hr exposure)

Precipitation conc:	With metabolic activation:	≥ 75 µg/mL		
	Without metabolic activation:	≥ 75 µg/mL		
Genotoxic effects:		+	?	-
	With metabolic activation:	[]	[]	[X]
	Without metabolic activation:	[]	[]	[X]
Method:	OECD Test Guideline 473 (1998)			
GLP:	Yes [X] No [] ? []			
Test Substance:	Bisphenol A-Bisimide (BPA-BI; CAS RN 54395-52-7; from General Electric Plastics); Purity: > 99.9%			
Remarks:	<u>Description of test procedure:</u> A preliminary toxicity assay was performed for the purpose of selecting doses for the chromosome aberration assay and consisted of an evaluation of test article effect on cell growth. CHO cells were seeded for each treatment condition at approximately 5x10 ⁵ cells/25 cm ² flask and were incubated at 37±1°C in a humidified atmosphere of 5±1% CO ₂ in air for 16-24 hours. Treatment was carried out by refeeding the flasks with complete medium for the non-activated study or S9 reaction mixture for the activated study, to which was added 50 µL dosing solution of test article in solvent or solvent alone. The osmolality in treatment medium of the solvent and of the highest test article concentration, the lowest precipitating test article concentration and the highest soluble test article concentration were measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. The cells were treated for 4 hours with and without S9, and continuously for 20 hours without S9. At completion of the 4-hour exposure period, the cells were washed refed with complete medium and returned to the incubator for a total of 20 hours from the initiation of treatment. At 20 hours after the initiation of treatment the cells were harvested. The presence of test article precipitate was assessed using the unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control. In the preliminary toxicity assay, the maximum dose tested was 1500 µg/mL. The test article was soluble in treatment medium at ≤ 15 µg/mL and precipitate was observed at ≥ 45 µg/mL. The osmolality of the test article concentrations in treatment medium were acceptable because they did not exceed the osmolality of the solvent by more than 20%. The pH of the highest concentration of test article in treatment medium was approximately 7.5. Based on the toxicity study, the doses chosen for the chromosome aberration assay were 0, 6.25, 12.5, 25, 35, 50, 75 and 100 µg/mL (4-hr treatment w/o S9 mix), 0, 6.25, 12.5, 25, 35, 50, 75, 100 and 125 µg/mL (20-hr treatment w/o S9 mix), and 0, 6.25, 12.5, 25, 50 and 75 µg/mL (4-hr treatment with S9 mix). Samples were run in duplicate, with and without metabolic activation.			
	<u>Selection of doses for microscopic analysis:</u> the first criterion, specified by the Guideline, is to select the highest dose with at least 50% reduction in cell growth or mitotic index relative to the solvent control with a sufficient number of scorable metaphase cells, regardless of test article precipitation in the treatment medium. For this study, the mitotic index was used to select the highest dose for each test condition. Two lower doses also were included.			

For the chromosome aberration assay, CHO cells were seeded and treated as described above. The osmolality in treatment medium of the solvent and of the highest test article concentration, the lowest precipitating test article concentration and the highest soluble test article concentration were measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. A concurrent toxicity test was conducted for each treatment. After cell harvest the cells were counted, test article precipitate was assessed and cell viability was determined by trypan blue dye exclusion. Cell counts and viability were used to determine cell growth inhibition.

The cells were exposed to the test article continuously for 4 or 20 hours in the non-activated study, and for 4 hours in the activated study. After the exposure period for the 4-hour exposure groups, the cells were washed and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid[®] was added to duplicate flasks for each treatment condition. Two hours after the addition of Colcemid[®], metaphase cells were harvested. Cells were collected approximately 20 hours after initiation of treatment. Slides were prepared from cells of each treatment and the cells stained with 5% Giemsa.

Evaluation of metaphase cells: The percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Initially, the non-activated and S9 activated 4-hour exposure groups were evaluated for chromosome aberrations and since a negative result was obtained in the non-activated 4-hour exposure group, the non-activated 20-hour continuous exposure group was then also evaluated for chromosome aberrations. When possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations. The number of metaphase spreads that were examined and scored per duplicate flask may have been reduced if the percentage of aberrant cells reached a statistically significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (≥ 10 aberrations) were also recorded. Chromatid and isochromatid gaps were recorded but not included in the analysis. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored.

Evaluation of test results: The toxic effects of treatment were based upon cell growth inhibition relative to the solvent-treated control and are presented for the toxicity and aberration studies. The number and

types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell were calculated and reported for each group. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's exact test at any test article dose group, the Cochran-Armitage test was used to measure dose-responsiveness. As a guide to interpretation of the data, the test article was considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). However, values that are statistically significant but do not exceed the range of historic solvent controls may be judged as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

Criteria for a Valid Test: The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control.

Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Negative and Positive controls: Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2 $\mu\text{g/mL}$. Cyclophosphamide (CP) was used as the positive control in the S9 activated study at final concentrations of 10 and 20 $\mu\text{g/mL}$. The solvent vehicle for the test article, dimethyl sulfoxide (DMSO), was used as the solvent control at the same concentration as that found in the test article-treated groups.

Results:

In the chromosome aberration assay, the test article was soluble in treatment medium at doses $\leq 50 \mu\text{g/mL}$ and precipitate was observed at $\geq 75 \mu\text{g/mL}$. The osmolality in the treatment medium of the highest concentration tested (125 $\mu\text{g/mL}$), was 401 mmol/kg. The osmolality in the treatment medium of the lowest precipitating concentration (75 $\mu\text{g/mL}$), was 397 mmol/kg. The osmolality in the treatment medium of the highest soluble concentration (50 $\mu\text{g/mL}$), was 394 mmol/kg. The osmolality of the solvent (DMSO) in treatment medium was 394 mmol/kg. The pH of the highest concentration of test article in treatment medium was approximately 7.0.

4-hour harvest without metabolic activation: No toxicity of BPA-BI was observed. The mitotic index at the highest dose evaluated for chromosome aberrations, 100 $\mu\text{g/mL}$, was 52% reduced relative to the solvent control. The doses selected for microscopic analysis were 25, 50 and 100 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not

significantly increased above that of the solvent control at any dose ($p \leq 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (16.0%) was statistically significant.

4-hour harvest with metabolic activation: No toxicity of BPA-BI was observed. The mitotic index at the highest dose evaluated for chromosome aberrations, 75 $\mu\text{g/mL}$, was 52% reduced relative to the solvent control. The doses selected for microscopic analysis were 12.5, 25, and 75 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose ($p \leq 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) treatment group (18.0%) was statistically significant.

20-hour harvest without metabolic activation: No toxicity of BPA-BI was observed. The mitotic index at the highest dose evaluated for chromosome aberrations, 50 $\mu\text{g/mL}$, was 51% reduced relative to the solvent control. The doses selected for microscopic analysis were 12.5, 25 and 50 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose ($p \leq 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (25.0%) was statistically significant.

Conclusion:

The positive and solvent controls fulfilled the requirements for a valid test. Under the conditions of the assay, BPA-BI was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in the presence and absence of S9 activation.

Summary of Test Results

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean ± SD)	Cells with Numerical Aberrations (%)	Cells with Structural Aberrations (%)
Vehicle (DMSO)	-	4	7.9	200	0.005 ± 0.071	3.0	0.5
Bisphenol A Bisimide (BPA-BI)							
25	-	4	7.5	200	0.010 ± 0.100	4.0	1.0
50	-	4	5.7	200	0.015 ± 0.122	3.5	1.5
100	-	4	3.8	200	0.015 ± 0.122	3.5	1.5
Positive control (MMC) 0.2	-	4	9.2	200	0.18 ± 0.434	2.5	16.0**
Vehicle (DMSO)	+	4	7.5	200	0.000 ± 0.000	3.5	0.0
Bisphenol A Bisimide (BPA-BI)							
12.5	+	4	7.6	200	0.010 ± 0.100	5.5	1.0
25	+	4	6.8	200	0.000 ± 0.000	4.5	0.0
75	+	4	3.6	200	0.010 ± 0.100	6.0	1.0
Positive control (CP) 10	+	4	5.7	100	0.300 ± 1.087	4.0	19.0**
Vehicle (DMSO)	-	20	9.1	200	0.000 ± 0.000	1.5	0.0
Bisphenol A Bisimide (BPA-BI)							
12.5	-	20	8.1	200	0.000 ± 0.000	1.5	0.0
25	-	20	8.5	200	0.010 ± 0.100	2.5	1.0
50	-	20	4.5	200	0.005 ± 0.071	1.5	0.5
Positive control (MMC) 0.1	-	20	8.1	100 ^a	0.280 ± 0.533	2.5	25.0**

Treatment: Cells from both the 4-hour and 20 hour treatment regimens were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: **, p≤0.01; using the Fisher's exact test.

^a Numerical aberrations are out of 200 cells scored.

Reference: Gudi, R. and M. Rao (2003) Unpublished report no. AA79BU.331.BTL entitled “*In vitro* mammalian chromosome aberration test” DRAFT dated November 11, 2003 for General Electric Company, Pittsfield, MA, USA; from BioReliance Corp., Rockville, MD, USA.

Reliability: (Klimisch Code 1) Valid without restrictions.

15.0 GENETIC TOXICITY *IN VITRO*

15.1 BACTERIAL TEST

15.1.1

Type: Bacterial reverse mutation assay (Ames test)
 System of testing: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538
 Concentrations: 0, 1, 10, 100, 500, 1000, 2500, 5000 and 10000 µg/plate
 Metabolic activation: With []; Without []; With and Without [X]; No data []
 Results: Negative

Cytotoxicity conc.:	No cytotoxicity observed with and without metabolic activation:
Precipitation conc.:	Not stated
Genotoxic effects:	With metabolic activation: positive []; ambiguous []; Negative [X] Without metabolic activation: positive []; ambiguous []; Negative [X]
Method:	Ames et al. (1975) Mutation Research 31:347-365 <u>Description of test procedure:</u> The plate test consisted of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Approximately 0.1 ml to 0.2 ml of the test organisms were treated with the test substance in the presence and absence of a metabolic activation system (Aroclor 1254-treated rat liver supernatant). One plate was used per concentration. The plates were incubated for approximately 48 hours at 37 °C, and scored for the number of colonies growing on each plate. <u>Solvent and Positive controls:</u> Deionized water was the solvent for the test substance and served as the solvent control. For the non-activation assay, the following positive control substances were used: Sodium azide (for strains TA1535 and TA100); 2-Nitrofluorene (for strains TA1538 and TA98); and 9-Aminoacridine (for strain TA1537). The positive control substance, 2-anthramine was used for all tester strains with metabolic activation. <u>Criteria for evaluating results:</u> The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. For strains TA1535, TA1537 and TA1538, a test substance producing a positive response equal to three times the solvent control value is considered mutagenic. For strains TA98 and TA100, a test substance producing a positive response equal to twice the solvent control value is considered mutagenic. In addition, a positive response must be repeated in a separate assay. <u>Activation system:</u> S9 liver homogenate prepared from Aroclor 1254-induced Sprague-Dawley male rats. The S9 mix was prepared fresh each day of testing.
Year:	1981
GLP:	Yes [] No [X] ? []
Test substance:	02-81-011535-014 AR #93479 (Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7) – Purity: See “Chemical Identity and Use Information” section.
Results:	The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains, was reported to be less than or approximately equal to the number of revertants in the solvent-treated negative control group, with and without metabolic activation.

Revertants Per Plate
Activation: None

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100
Solvent (distilled water)	14	5	16	36	123
Solvent (distilled water)	16	9	19	56	128
1	6	17	15	55	151
10	16	7	23	50	113
100	16	10	18	51	138
500	17	10	22	53	122
1000	15	8	23	61	170
2500	18	13	12	47	160
5000	19	19	15	63	126
10000	10	8	14	61	137
Positive Control	584	190	820	876	1143
Positive Control	645	299	925	913	1249

Revertants Per Plate
Activation: Rat Liver S9

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100
Solvent (distilled water)	11	11	24	69	141
Solvent (distilled water)	12	18	27	73	143
1	17	11	20	66	132
10	11	10	23	62	157
100	11	19	24	61	155
500	9	10	17	69	153
1000	15	7	18	62	161
2500	13	8	13	47	188
5000	12	12	23	65	172
10000	12	9	12	66	162
Positive Control	443	227	1555	1510	1608
Positive Control	450	308	1756	1554	1661

Conclusion: The test substance did not exhibit mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference: Jagannath, D. R. and D. J. Brusick (1981) Unpublished report for Project No. 20988 entitled "Mutagenicity Evaluation of 02-81-011535-014 AR #93479" dated April 1981 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc., Kensington, MD, USA.

Reliability: (Klimisch Code 2) Valid with restrictions. Acceptable study report that meets basic scientific principles.

15.1.2

Type: Bacterial reverse mutation assay (Ames test)
System of testing: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538; *Saccharomyces cerevisiae* strain D4
Concentrations: 0, 0.1, 1.0, 10, 100 and 500 µg/plate (TA98, TA100, TA1535, TA1537, TA1538, and D4); and 500, 1000, 2000 µg/plate (TA100) – second test

Metabolic activation:	With []; Without []; With and Without [X]; No data []
Results:	Negative
Cytotoxicity conc.:	With metabolic activation: not stated Without metabolic activation: not stated
Precipitation conc.:	None
Genotoxic effects:	With metabolic activation: positive []; ambiguous []; Negative [X] Without metabolic activation: positive []; ambiguous []; Negative [X]
Method:	Ames et al. (1975) Mutation Research 31:347-365 <u>Description of test procedure:</u> The plate test consisted of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Approximately 10^8 cells were treated with the test substance in the presence and absence of a metabolic activation system (Aroclor 1254-treated rat liver supernatant). One plate was used per concentration. The plates were incubated for 48 hours at 37 °C, and scored for the number of colonies growing on each plate. <u>Solvent and Positive controls:</u> Dimethylsulfoxide (DMSO) was the solvent for the test substance and served as the solvent control. For the non-activation assay, the following positive control substances were used: Methylnitrosoguanidine (for strains TA1535, TA100 and D4); 2-Nitrofluorene (for strains TA1538 and TA98); and quinacrine mustard (for strain TA1537). The positive control substances, 2-anthramine (strains TA1535 and TA100), 2-acetylaminofluorene (strains TA1538 and TA98) and 8-aminoquinoline (strain TA1537) were used with metabolic activation. The positive control substance used for D4 without activation was not identified in the report. <u>Criteria for evaluating results:</u> The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. For strains TA1535, TA1537 and TA1538, if the solvent control value is within the normal range, a test substance producing a positive response over three concentrations with the lowest increase equal to twice the solvent control is considered mutagenic. For strains TA98, TA100 and D4, a test substance producing a positive response over three concentrations with the lowest increase equal to twice the solvent control (TA100) or two to three times the solvent control (TA98 and D4) is considered mutagenic. In addition, a positive response must be repeated in a separate assay. <u>Activation system:</u> S9 liver homogenate prepared from Aroclor 1254-induced male Sprague-Dawley rats.
Year:	1977
GLP:	Yes [] No [X] ? []
Test substance:	2,2-Bis[4-(3,4-dicarboxyphenoxy)phenyl]propane-bis-N-methyl imide (BPA-Bisimide; 09-77-011154-022) – Purity: See “Chemical Identity and Use Information” section.
Results:	The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains, except TA100 in the first test, was reported to be less than or approximately equal to the number of revertants in the solvent-treated

negative control group, with and without metabolic activation. Doses of 1000 and 2000 µg/plate were used with TA-100 and the test was repeated because of an increased mutation frequency observed at 500 µg/plate. The results of the second test were negative.

Revertants Per Plate
Activation: None

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4*
Solvent (DMSO)	10	18	25	31	169 235	32
0.1	10	22	20	28	187 ---	46
1.0	16	15	19	33	216 ---	37
10	14	25	19	28	257 ---	33
100	12	18	21	27	216 ---	39
500	9	15	10	31	304 268	41
1000	--	--	--	--	--- 213	--
2000	--	--	--	--	--- 217	--
Positive Control	653	564	>1000	>1000	>1000	573

Revertants Per Plate
Activation: Rat Liver S9

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4*
Solvent (DMSO)	17	13	24	35	267 205	23
0.1	15	13	20	48	220 ---	
1.0	10	12	24	34	242 ---	20
10	12	27	29	36	228 ---	20
100	5	10	17	27	264 ---	19
500	11	14	24	39	316 210	22
1000	--	--	--	--	--- 246	21
2000	--	--	--	--	--- 269	--
Positive Control	131	213	574	891	831	48--

Conclusion: The test substance did not exhibit mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference: Jagannath, D.R. and D. J. Brusick (1977) Unpublished report for Project No. 20838 entitled "Mutagenicity evaluation of 09-77-011154-022" dated November 1977 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc., Kensington, MD, USA.

Reliability: (Klimisch Code 2) Valid with restrictions. Acceptable study report that meets basic scientific principles.

15.2 NON-BACTERIAL *IN VITRO* TEST (MAMMALIAN CELLS)

Type: *In vitro* mammalian cell gene mutation test (Mouse lymphoma assay)
System of testing: Mouse lymphoma L5178Y cells
Concentration: 0, 10, 25, 50, 75 and 100 µg/mL with and without activation (4-hour exposure)
 0, 5, 10, 25, 50, and 75 µg/mL without activation (24-hour exposure)
Metabolic activation: With []; Without []; With and Without [X];

Results:	No data [] Negative without metabolic activation with a 24-hour exposure and with metabolic activation with a 4-hour exposure; equivocal without metabolic activation with a 4-hour exposure		
Cytotoxicity conc.:	With metabolic activation:	None	
	Without metabolic activation:	≥ 500 µg/mL	
Genotoxic effects:		+	? -
	With metabolic activation:	[]	[] [X]
	Without metabolic activation:	[]	[] [X]
Method:	OECD Test Guideline 476 (1998)		
GLP:	Yes [X] No [] ? []		
Test Substance:	Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7; from General Electric Plastics); Purity: > 99.9%		
Remarks:	<p><u>Description of test procedure:</u> The preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.15 to 1500 µg/mL in both the absence and presence of S9-activation with a 4-hour exposure and without activation with a 24-hour exposure. Cell population density was determined 24 and 48 hours after the initial exposure to the test article. The cultures were adjusted to 3x10⁵ cells/mL after 24 hours only. Toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures after 48 hours.</p> <p>The mutagenesis assay was carried out by combining 6x10⁶ L5178Y/TK^{+/-} cells, medium or S9 activation mixture and 100 µL dosing solution of test or control article in solvent or solvent alone and incubated for 4 (with and without activation) or 24 (without activation) hours. The positive controls were treated with MMS (at 10 and 20 µg/mL for the 4-hour exposure or 2.5 and 5.0 µg/mL for the 24-hour exposure) and 7,12-DMBA (2.5 and 4 µg/mL).</p> <p><u>Expression of the mutant phenotype:</u> For expression of the mutant phenotype, the cultures were counted and adjusted to 3x10⁵ cells/mL at approximately 24 and 48 hours after treatment. Cultures with less than 3x10⁵ cells/mL were not adjusted. For expression of the TK^{-/-} cells, two flasks per culture were cloned for TFT (trifluorothymidine, the selective agent) or VC (viable count). The cells were diluted in cloning medium to concentrations of 3x10⁶ cells/100/mL for the TFT flask and 600 cells/100mL for the VC flask. Cells were plated and incubated for 10-14 days.</p> <p><u>Scoring procedures:</u> The VC plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were counted for each culture with ≥ 20% total relative growth (including at least one concentration with ≥ 10% but ≤ 20% total growth). The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of approximately 0.2 to 1.1 mm.</p>		

Evaluation of results: The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 10^6 surviving cells) was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding VC plates and multiplying by the dilution factor (2×10^{-4}) then multiplying by 10^6 . In evaluation of the data, increases in mutant frequencies that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. The following criteria are presented as a guide to interpretation of the data: (1) A result was considered positive if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of ≥ 100 mutants per 10^6 clonable cells over the background level, (2) A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level, (3) A result was considered negative if the mutant frequency in treated cultures was fewer than 55 mutants per 10^6 clonable cells over the background level.

Criteria for a Valid Test: For the negative control, the spontaneous mutant frequency of the cultures must be within 20 to 120 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%. For positive controls, at least one concentration of each positive control must exhibit mutant frequencies of ≥ 100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies. For the BPA-BI cultures, a minimum of four analyzable concentrations with mutant frequency data was required.

Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Results:

The maximum dose tested in the preliminary toxicity assay was 1500 $\mu\text{g/mL}$. Visible precipitate was present at $\geq 150 \mu\text{g/mL}$ in treatment medium. No visible precipitate was present at concentrations of $\leq 50 \mu\text{g/mL}$ in treatment medium. The osmolality of the solvent control was 462 mmol/kg and the osmolality of the highest soluble dose, 50 $\mu\text{g/mL}$, was 439 mmol/kg. Suspension growth relative to the solvent controls was 21% without activation and a 4-hour exposure, 73% with S9 activation and a 4-hour exposure, and 50% without activation and a 24-hour exposure at 1500 $\mu\text{g/mL}$. Based on the results of this preliminary test the concentrations selected for the mutagenesis assay were 0, 10, 25, 50, 75 and 100 $\mu\text{g/mL}$ with and without activation for the 4-hour exposure and 0, 5, 10, 25, 50 and 75 $\mu\text{g/mL}$ for the 24-hour exposure without activation.

Results for cultures treated for four hours (initial assay): Visible precipitate was present at a concentration of 100 µg/mL. No visible precipitate was observed at concentrations ≤ 75 µg/mL. In the non-activated system, suspension growth ranged from 73 to 101%. In the activated system, suspension growth ranged from 92 to 111%. One non-activated cloned culture (4-hour exposure) exhibited a mutant frequency of ≥ 100 mutants per 10^6 clonable cells greater than the solvent control. Two non-activated and one activated cloned cultures (4-hour exposure) exhibited mutant frequencies between 55 and 99 mutants per 10^6 clonable cells. A dose-response was not observed in either non-activated or activated systems. The total growths ranged from 37 to 108% for the non-activated cultures at concentrations of 10 to 100 µg/mL and 40 to 87% for the S9-activated cultures at concentrations of 10 to 100 µg/mL. The results of the initial 4-hour exposure assay in the non-activated system were considered equivocal and negative in the activated system. Because no unique metabolic requirements were known about the test article, only an extended treatment assay was performed in the absence of S9 for a 24-hour exposure period.

Results for cultures treated for 24 hours (extended treatment assay): Visible precipitate was present at 75 µg/mL. No visible precipitate was observed at concentrations ≤ 50 µg/mL. Cultures treated with concentrations of 0, 5, 10, 25, 50 and 75 µg/mL were cloned and produced a range in suspension growth of 57 to 98%. No cloned culture exhibited a mutant frequency of between 55 and 99 mutants per 10^6 clonable cells over that of the solvent control. A dose-response trend was not observed. The total growths ranged from 53 to 156% at concentrations of 5 to 75 µg/mL. The TFT-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

**Cloning Data for L5178Y/TK⁺ Mouse Lymphoma Cells Treated with BPA-BI
in the Absence of Exogenous Metabolic Activation
Initial Assay (4-hour exposure)**

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
		Counts			Mean	Counts			Mean			
0 (solvent)	1	60	60	30	50 ± 14	157	80	210	149 ± 53	67	--	--
0 (solvent)	2	39	18	+	29 ± 9	171	225	122	173 ± 42	33	--	--
Mean Solvent Mutant Frequency = 50												
10	A	54	65	32	50 ± 14	181	164	170	172 ± 7	59	9	108
10	B	21	28	30	26 ± 4	210	165	166	180 ± 21	29	-21	107
25	A	57	47	66	57 ± 8	160	107	155	141 ± 24	81	31	82
25	B	24	27	26	26 ± 1	170	156	109	145 ± 26	35	-15	82
50	A	92	55	71	73 ± 15	153	194	175	174 ± 17	84	33	98
50	B	53	65	51	56 ± 6	65	156	94	105 ± 38	107	57	59
75	A	66	53	63	61 ± 6	179	109	179	156 ± 33	78	28	84
75	B	90	93	67	83 ± 12	67	132	146	115 ± 34	145	95	61
100*	A	77	78	89	81 ± 5	190	157	175	174 ± 13	93	43	91
100*	B	76	73	51	67 ± 11	48	135	64	82 ± 38	162	112	37
Positive Control - Methyl Methanesulfonate (µg/mL)												
10	--	57	51	74	61 ± 10	27	114	116	86 ± 41	142	92	37
20	--	106	55	65	75 ± 22	18	47	45	37 ± 13	411	361	9

Solvent = DMSO

* Precipitating concentration

+ = Culture lost

^a Mutant frequency (per 10⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200^b Induced mutant frequency (per 10⁶ surviving cells) = mutant frequency - average mutant frequency of solvent controls^c % Total growth = (% suspension growth x % cloning growth) / 100

**Cloning Data for L5178Y/TK⁺ Mouse Lymphoma Cells Treated with BPA-BI
in the Presence of Exogenous Metabolic Activation
Initial Assay (4-hour exposure)**

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
		Counts			Mean	Counts			Mean			
0 (solvent)	1	48	32	13	31 ± 14	122	175	178	158 ± 26	39	--	--
0 (solvent)	2	21	13	+	17 ± 3	170	140	90	133 ± 33	25	--	--
Mean Solvent Mutant Frequency = 32												
10	A	35	24	47	35 ± 9	113	87	110	103 ± 12	68	36	76
10	B	18	31	21	23 ± 6	74	76	113	88 ± 18	53	21	66
25	A	52	32	18	34 ± 14	164	117	77	119 ± 36	57	25	86
25	B	40	18	30	29 ± 9	160	84	112	119 ± 31	49	17	87
50	A	42	47	25	38 ± 9	170	100	67	112 ± 43	68	35	81
50	B	18	15	52	28 ± 17	66	77	116	86 ± 21	66	33	60
75	A	+	58	38	48 ± 8	92	61	117	90 ± 23	107	74	61
75	B	18	26	22	22 ± 3	42	53	70	55 ± 12	80	48	40
100*	A	45	17	31	31 ± 11	143	81	86	103 ± 28	60	28	65
100*	B	24	21	+	23 ± 1	100	84	173	119 ± 39	38	5	79
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)												
2.5	--	78	86	+	82 ± 3	57	64	96	72 ± 17	227	194	32
4	--	177	195	130	167 ± 27	77	100	120	99 ± 18	338	306	26

Solvent = DMSO

* Precipitating concentration

+ = Culture lost

^a Mutant frequency (per 10⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200^b Induced mutant frequency (per 10⁶ surviving cells) = mutant frequency - average mutant frequency of solvent controls^c % Total growth = (% suspension growth x % cloning growth) / 100

**Cloning Data for L5178Y/TK⁺ Mouse Lymphoma Cells Treated with BPA-BI
in the Absence of Exogenous Metabolic Activation
Extended Treatment Assay (24-hour exposure)**

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq. ^a	Induced Mutant Freq. ^b	% Total Growth ^c
		Counts			Mean	Counts			Mean			
0 (solvent)	1	101	52	53	69 ± 23	210	166	137	171 ± 30	80	--	--
0 (solvent)	2	44	54	76	58 ± 13	203	100	142	148 ± 42	78	--	--
Mean Solvent Mutant Frequency = 79												
5	A	42	33	90	55 ± 25	181	139	116	145 ± 27	76	-4	82
5	B	58	96	91	82 ± 17	111	135	143	130 ± 14	126	47	78
10	A	47	74	78	66 ± 14	137	166	143	149 ± 12	89	10	53
10	B	45	32	+	39 ± 5	112	105	204	140 ± 45	55	-24	86
25	A	80	61	120	87 ± 25	240	227	270	246 ± 18	71	-8	126
25	B	46	98	81	75 ± 22	244	271	234	250 ± 16	60	-19	144
50	A	84	46	74	68 ± 16	249	159	196	201 ± 37	68	-12	115
50	B	51	40	126	72 ± 38	130	137	256	174 ± 58	83	4	93
75*	A	91	76	54	74 ± 15	281	263	219	254 ± 26	58	-21	156
75*	B	40	38	77	52 ± 18	222	212	236	223 ± 10	46	-33	135
Positive Control - Methyl Methanesulfonate (µg/mL)												
2.5	--	76	266	123	155 ± 81	113	107	124	115 ± 7	270	191	67
5	--	242	150	275	222 ± 53	92	124	90	102 ± 16	436	357	44

Solvent = DMSO

* Precipitating concentration

+ = Culture lost

^a Mutant frequency (per 10⁶ surviving cells) = (Average # TFT colonies / average # VC colonies) x 200^b Induced mutant frequency (per 10⁶ surviving cells) = mutant frequency - average mutant frequency of solvent controls^c % Total growth = (% suspension growth x % cloning growth) / 100

Reference:

Reliability:

Conclusions: All criteria for a valid study were met as described in the protocol. The results of the L5178Y/TK⁺ Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, the mutagenicity of BPA-BI was concluded to be negative without activation with a 24-hour exposure and with activation with a 4-hour exposure. The 4-hour exposure without activation was equivocal based on the criteria established in the protocol (Author of the Report). However, this response occurred at precipitating concentrations and was not repeated in the more stringent 24-hour test. Therefore, BPA-BI is considered to be negative in this assay (Sponsor of the Study).

San, R. H. C. and J. J. Clarke (2003) Unpublished report no AA79BU.704.BTL entitled “*In vitro* mammalian cell gene mutation test (L5178Y/TK⁺ mouse lymphoma assay)” DRAFT dated October 31, 2003 for General Electric Company, Pittsfield, MA, USA; from BioReliance Corp., Rockville, MD, USA.

(Klimisch Code 1) Valid without restrictions.

16.0 REPEATED DOSE TOXICITY**16.1**

Species/Strain: Rat/Sprague-Dawley Crl:COBS®, CD®, (SD) Br
 Sex: Female ☐; Male ☐; Male/Female ☒; No data ☐
 Route of Administration: Oral, Dietary feed
 Exposure Period: 30 days
 Frequency of Treatment: Daily
 Post Exposure
 Observation Period: None
 Dose: 0, 1, 2, and 4% of BPA-BI in basal diet (approximately 0, 646 – 765, 1277 – 1490, and 2750 – 3160 mg/kg/day, respectively)
 Control Group: Yes ☒ No ☐ No data ☐
 Concurrent no treatment ☒ Concurrent vehicle ☐ Historical ☐
 NOAEL: 4% (approximately 2750 to 3160 mg/kg/day)
 LOAEL: > 4%
 Method: Groups of 10 rats/sex were fed BPA-BI at concentrations of 0, 1, 2 and 4%. All rats were observed for mortality twice each day. Clinical signs and body weights were recorded at initiation and weekly thereafter. Food consumption was recorded weekly. After 31 days of treatment, all surviving rats were weighed, killed and a gross necropsy was performed. At necropsy, the liver and kidneys of each animal were weighed and organ to body weight ratios determined. The following tissues were preserved from all animals: brain, pituitary, thoracic spinal cord, eyes, salivary glands, thyroid, parathyroids, thymus, trachea, esophagus, lung, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, duodenum, jejunum, ileum, colon, cecum, mesenteric lymph node, urinary bladder, testes with epididymides and prostate (males), ovaries and uterus (females), femur, costal bone marrow, skeletal muscle, and all gross lesions. Microscopic evaluation was conducted on sections of the lungs, liver, brain and kidneys from rats of all treatment groups. Reproductive organs were not evaluated histologically. The following statistical tests were utilized to evaluate body weight changes, total food consumption and organ weights: Bartlett's test for homogeneity of variance and one-way classification analysis of variance (ANOVA). Since the ANOVA proved to be not significant for all of the analyses, no other tests were performed. All analyses were performed at the 5% one-tailed probability level.

Year: 1982
 GLP: Yes ☒ No ☐ ? ☐
 Test Substance: Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); Lot UI-82-3 from General Electric Company; Purity: See "Chemical Identity and Use Information" section.

Results: No deaths occurred during the study. No compound-related clinical observations were noted throughout the study. Body weight and food consumption data of the compound-treated males and females were generally comparable to those of their respective controls. Individual and mean terminal body weights, absolute organ weights and organ weights relative to terminal body weight were not affected by treatment. No compound-related organ or tissue changes were evident macroscopically or microscopically.

Reference: Burdock, G. A. and W. Kundzins (1982) Unpublished report number 349-262 entitled “Thirty-Day Subchronic Oral Toxicity Study in Rats, BPA-BI and BPA-DA” dated December 17, 1982 for General Electric Company, Mount Vernon Indiana, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and
 Burdock, G. A. (1984) Unpublished addendum to final report number 349-262 entitled “Thirty-Day Subchronic Oral Toxicity Study in Rats, BPA-BI and BPA-DA” dated December 22, 1984 for General Electric Company, Mount Vernon Indiana, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.

Reliability: (Klimisch Code 2) Valid with restrictions. Minimal data collected for a repeat dose study.

17.0 REPRODUCTIVE TOXICITY

No studies were found. Study according to OECD Guideline 421 in progress.

18.0 DEVELOPMENTAL TOXICITY/TERATOGENICITY

18.1

Species/Strain: Rabbit, New Zealand White
 Sex: Female [X]; Male []; Male/Female []; No data []
 Route of Administration: Oral (gavage)
 Duration of Test: 29 days
 Exposure Period: Days 6 through 18 of gestation
 Frequency of Treatment: Daily
 Dose: 1000 mg/kg/day
 Control group: Yes [X] No [] No data []
 Concurrent no treatment [] Concurrent vehicle [X] Historical []
 Positive Control (thalidomide)

NOEL Maternal
 Toxicity: > 1000 mg/kg/day
 NOEL Teratogenicity: > 1000 mg/kg/day
 Year: 1983
 GLP: Yes [X] No [] ? []
 Test Substance: Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); Lot UI-82-3 from General Electric Company; Purity: See “Chemical Identity and Use Information” section.

Method: Ninety mature New Zealand White female rabbits were obtained from Dutchland Laboratory Animals, Inc., Denver, PA for use in this study. The animals were acclimated for a minimum of 22 days prior to the initiation of the study. During the period of acclimation, the rabbits were examined for general health and appearance. The animals were uniquely identified by ear tag and provided commercial rabbit ration (Purina lab Rabbit Chow®) and tap water *ad libitum*. The environment of the study room was maintained at 70-78 °C, relative humidity of 53-86% and a 12-hour light/dark cycle. At Day 0 of gestation, the body weights ranged from 2845 to 4700 grams. The animals were artificially inseminated with sperm from the laboratory breeding stock five hours after induction of ovulation with chorionic gonadotropin. Five groups were included in this study; for the purposes of this summary, only three groups (control, positive control, and BPA-BI treated) will be discussed. Sixteen animals per group (to obtain at least 12 pregnant) were treated with vehicle (0.5%

carboxymethyl cellulose), positive control (thalidomide; 150 mg/kg/day) or BPA-BI (1000 mg/kg/day). Thalidomide and BPA-BI were suspended in vehicle to provide dose volumes of 1.5 and 4.0 ml/kg, respectively. Control dose volume was 4.0 ml/kg. The dose was administered from gestation day (gd) 6 through 18, approximately the same time each day, and was based on each individual body weight on gd 6 (starting on gd 11, two animals in the control group, four animals in the thalidomide group and five animals in the BPA-BI group were dosed based on gd 11 body weight). All of the animals were observed daily for mortality, moribundity and clinical signs. Body weights were recorded on gd 0, 6, 11, 15, 19, and 29. Individual food consumption was recorded weekly. On gd 29, the animals were sacrificed, examined for gross pathology of the external surface and viscera, and the uterus excised and weighed. The fetuses were taken by cesarean section and the following recorded for each litter: the number of corpora lutea per ovary; the number and placement of uterine implantation sites; live and dead fetuses; early and late resorptions; and any other abnormalities. Fetuses were removed from the placenta, individually identified, examined externally, weighed and measured from the frontal-parietal suture to the base of the tail (crown-rump distance). Cesarean sections were also performed on dams that were found dead, sacrificed moribund or sacrificed due to early delivery. The number of corpora lutea, implantations, resorptions and live or dead fetuses was recorded.

Visceral Examination of Fetuses: The unfixed fetuses underwent visceral examination according to the method of Staples. All of the fetuses were opened by longitudinal incision, the sex determined and examined grossly both externally and internally. Major organs were inspected *in situ* with special attention to the heart and major blood vessels. The heads of approximately one-third of the fetuses were removed, fixed in Bouin's solution, sectioned by Wilson's freehand sectioning technique for examination of the eyes, palate, nasal septum and brain. The prepared sections were then re-examined against a light box with the aid of magnification.

Skeletal Examination of Fetuses: Following visceral examination, all fetuses (minus the head for approximately one-third of the fetuses) were eviscerated and placed in 95% ethyl alcohol. After fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution. The skull, vertebral column, rib cage, pectoral and pelvic girdles, long bones and extremities of each skeleton were examined for degree of ossification, bone alignment, and possible anomalies. Examinations were performed with the aid of magnification on a light box.

Statistical Analyses: Mean maternal body weight changes, food consumption, percentage data (implantations, resorptions and males), and fetal viability were analyzed in the following order: Levene's test for homogeneity of variance; if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA); if the variance proved to be heterogeneous, a series of transformations was performed until homogeneity was achieved followed by ANOVA. If ANOVA was significant, the Games and Howell modification of the Tukey-Kramer honestly significant difference test was used to compare groups. Pregnancy rates were analyzed by Fisher's exact test. External,

visceral, and skeletal anomalies were evaluated by a multiple proportions test. Analysis of covariance (ANCOVA) was used to analyze mean fetal weights and lengths with the litter used as the experimental unit. Levene's test and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. treatment group mean comparisons were evaluated at the 5% two-tailed probability level.

Range-finding study: A range-finding study was conducted to select the dose used in this study. Four non-pregnant New Zealand White rabbits were dosed with BPA-BI at 2000 mg/kg/day for the first six days and, following a three-day rest period, the dose was changed to 1000 mg/kg/day for the remaining seven days of the study. One of the four rabbits died on Day 4. Compound-related clinical signs included depression, slight depression and anorexia. Two of the surviving animals lost weight throughout the study. Based on this study, 1000 mg/kg/day was selected for the teratology study.

Results:

Weight loss was observed in the thalidomide-treated group during the treatment period. Statistical evaluation of body weight change did not, however, reveal any significant differences between treated and control groups. No effects on food consumption or gross pathology of the dams were observed. The following tables summarize the fetal results:

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-BI (1000 mg/kg/day)
Number of dams	16	16	16
Number pregnant	14	16	16
Pregnancy rate (%)	88	100	100
Number dams surviving to gd 29 (survival rate)	13 (93%)	15* (100%)	15 (94%)
Mean number of			
Corpora lutea	13.4	12.2	10.5
Implantations	9.4	8.3	7.8
Resorptions-total	1.2	5.3	1.1
Fetuses – live	7.5	3.4	6.4
– dead	0.5	0	0
Indices (mean per litter)			
Implantation efficiency (%)	73.6	68.1	73.1
Incidence of resorption (%)	17.2	61.0	17.4
Incidence of fetal mortality (%)	3.8	0	0
Incidence of fetal viability (%)	79.2	39.1	82.6
Live fetuses			
Mean body weight (g) – males	40.91	38.36	44.04
– females	39.90	37.60	44.57
Mean length (cm) – males	9.49	9.03	9.48
– females	9.33	8.92	9.63
Percent Males	51.5	58.0	47.8
Mean uterine weights – gravid (g)	485.3	228.3	406.5

* One animal died accidentally on gd 8

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-BI (1000 mg/kg/day)
External			
# of litters examined	12	11	14
# of litters with anomalous fetuses	2	10*	0
% of litters with anomalous fetuses	16.7	90.9	0
Mean values (per litter)			
# of fetuses with variants	0	0.5	0
Incidence of variants (%)	0	14.4	0
# of fetuses with anomalies	0.3	2.7	0
Incidence of anomalies (%)	2.4	64.1	0
Visceral – Fetal Heads			
# of litters examined	12	9	13
# of litters with anomalous fetuses	0	3	0
% of litters with anomalous fetuses	0	33.3	0
Mean values (per litter)			
# of fetuses with variants	0	0.1	0
Incidence of variants (%)	0	3.7	0
# of fetuses with anomalies	0	0.4	0
Incidence of anomalies (%)	0	16.7	0
Visceral – Torso and Limbs			
# of litters examined	12	11	14
# of litters with anomalous fetuses	0	8*	1
% of litters with anomalous fetuses	0	72.7	7.1
Mean values (per litter)			
# of fetuses with variants	0.8	2.9	0.6
Incidence of variants (%)	11.1	63.9	9.3
# of fetuses with anomalies	0	1.5	0.1
Incidence of anomalies (%)	0	38.8	1.8
Skeletal – Skulls			
# of litters examined	12	11	14
# of litters with anomalous fetuses	0	2	0
% of litters with anomalous fetuses	0	18.2	0
Mean values (per litter)			
# of fetuses with variants	0.5	1.6	0.1
Incidence of variants (%)	11.3	60.9	3.4
# of fetuses with anomalies	0	0.2	0
Incidence of anomalies (%)	0	11.4	0
Skeletal – Torso and Limbs			
# of litters examined	12	11	14
# of litters with anomalous fetuses	0	10*	0
% of litters with anomalous fetuses	0	90.9	0
Mean values (per litter)			
# of fetuses with variants	0.6	3.9	0.4
Incidence of variants (%)	6.9	91.7	5.0
# of fetuses with anomalies	0	2.2	0
Incidence of anomalies (%)	0	54.4	0

* Statistically significantly different from vehicle control group ($p < 0.05$)

Conclusion: There were no differences from control in the thalidomide or BPA-BI dose groups for maternal, ovarian or uterine data. The thalidomide-treated group exhibited changes consistent with the known teratogenic effect of this compound. The thalidomide group may additionally have had an increase in resorptions and exhibited a possible fetotoxic effect as demonstrated by slightly decreased mean body weights and lengths of the fetuses. There were no effects on any fetal parameters from BPA-BI treatment. Based on the results of this study, BPA-BI is not a developmental toxin.

Reference: Burdock, G. A. (1983) Unpublished report number 349-267 entitled “Teratogenicity Study in Rabbits, PI, BPA-BI, BPA-DA” dated August 25, 1983 for General Electric Company, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and Burdock, G. A. (1982) Unpublished report no 349-263 entitled “Two-Week Pilot Toxicity Study in Rabbits: BPA-BI, BPA-DA, PI, and 4-NPI” dated August 20, 1982 for General Electric Company, Mount Vernon, IN, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.

Reliability: (Klimisch Code 1) Reliable without restrictions.

18.2

Species/Strain: Rat; Crl:CD[®](SD)BR
Sex: Female [X]; Male []; Male/Female []; No data []
Route of Administration: Oral (gavage)
Duration of Test: 20 days
Exposure Period: Days 6 through 15 of gestation
Frequency of Treatment: Daily
Dose: 1000 mg/kg/day
Control group: Yes [X] No [] No data []
 Concurrent no treatment [] Concurrent vehicle [X] Historical []

NOEL Maternal

Toxicity: > 1000 mg/kg/day

NOEL Teratogenicity: > 1000 mg/kg/day

Year: 1987

GLP: Yes [X] No [] ? []

Test Substance: Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); from General Electric Company; Purity: See “Chemical Identity and Use Information” section.

Method: One hundred twenty, successfully mated Sprague-Dawley female rats, obtained from Charles River Breeding Laboratories, Inc., (Portage, MI), were used in this study. Prior to in-house breeding, the rats were examined for general health and appearance. The animals were uniquely identified by ear tag and provided commercial rat ration (Purina Certified Rodent Chow[®]) and tap water *ad libitum*. The environment of the study room was monitored daily and a 12-hour light/dark cycle was used. Animals were mated one male to one female for 17 days. The day that vaginal sperm or a copulation plug was observed was designated Day 0 of gestation. At Day 0 of gestation, the body weights ranged from 195 to 289 grams. Five groups were included in this study; for the purposes of this summary, only two groups (control, and BPA-BI-treated) will be discussed.

Twenty-four animals per group were treated with vehicle (0.5% carboxymethyl cellulose) or BPA-BI (1000 mg/kg/day). Dose volume was 10.0 ml/kg. The dose was administered from gestation day (gd) 6 through 15, approximately the same time each day, and was based on the most recently recorded body weight. All of the animals were observed daily for mortality, moribundity and clinical signs. Body weights and food consumption were recorded on gd 0, 6, 8, 12, 16, and 20. On gd 20, the animals were sacrificed, examined for gross pathology of the external surface and viscera, and the uterus excised and weighed. The fetuses were taken by cesarean section and the following recorded for each litter: the number of corpora lutea per ovary; the number and placement of uterine implantation sites; live and dead fetuses; early and late resorptions; and any other abnormalities. Fetuses were removed from the placenta, individually identified, examined externally, and weighed.

Visceral Examination of Fetuses: Approximately one-third of the live fetuses were selected for visceral examination according to the method of Wilson.

Skeletal Examination of Fetuses: The remaining fetuses were eviscerated and placed in 95% ethyl alcohol. After fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution. The skull, vertebral column, rib cage, pectoral and pelvic girdles, long bones and extremities of each skeleton were examined for degree of ossification, bone alignment, and possible anomalies.

Statistical Analyses: Mean maternal body weight changes, food consumption, percentage data (implantations, resorptions and males), and fetal viability were analyzed in the following order: Levene's test for homogeneity of variance; if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA); if the variance proved to be heterogeneous, a series of transformations was performed until homogeneity was achieved followed by ANOVA. If ANOVA was significant, the Dunnett's test was used to compare groups. Pregnancy rates, clinical observations and fetal skeletal observations were analyzed by Cochran-Armitage and Fisher-Irwin Exact Tests. Analysis of covariance (ANCOVA) was used to analyze mean fetal weights with the litter used as the experimental unit. Levene's test and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. treatment group mean comparisons were evaluated at the 5% two-tailed probability level.

Range-finding study: A range-finding study was conducted to select the dose used in this study. Five pregnant Sprague-Dawley rats were dosed with BPA-BI at doses of 150, 400 or 1000 mg/kg/day from days 6 through 15 of gestation. No maternal or fetal effects were observed at any dose. Based on this study, 1000 mg/kg/day was selected for the teratology study.

Results:

No treatment-related effect on maternal body weight or clinical observations were observed in the BPA-BI treated group. No effects on food consumption or gross pathology of the dams were observed. The following tables summarize the fetal results:

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
Number of dams	24	24
Number pregnant	23	24
Pregnancy rate (%)	96	100
Number dams surviving to gd 29 (survival rate)	24 (100%)	24 (100%)
Mean number of Corpora lutea	16.9	17.2
Implantations (% Efficiency)	14.8 (89)	14.9 (88)
Resorptions-total	0.9	0.7
Fetuses – live	14.0	14.3
– dead	0	0
Live fetuses		
Mean body weight (g) – males	3.6	3.6
– females	3.3	3.5
Mean uterine weights – gravid (g)	76.3	78.3

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
External Variations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	3	2
% of litters with anomalous fetuses	13	8.3
Fetal Incidence		
# of fetuses with variants	4	2
Incidence of variant (%)	1.2	0.6
External Malformations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	1	0
% of litters with anomalous fetuses	4.3	0
Fetal Incidence		
# of fetuses with variants	1	0
Incidence of variant (%)	0.3	0
Soft Tissue Variations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	6	14
% of litters with anomalous fetuses	26	58
Fetal Incidence		
# of fetuses with variants	9	22
Incidence of variant (%)	9.2	21
Soft Tissue Malformations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	0	1
% of litters with anomalous fetuses	0	4.2
Fetal Incidence		
# of fetuses with variants	0	1
Incidence of variant (%)	0	1.0
Skeletal Variations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	23	24
% of litters with anomalous fetuses	100	100
Fetal Incidence		
# of fetuses with variants	120	127
Incidence of variant (%)	54	54

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
Skeletal Malformations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	1	0
% of litters with anomalous fetuses	4.3	0
Fetal Incidence		
# of fetuses with variants	1	0
Incidence of variant (%)	0.4	0

Conclusion: There were no differences from control in the BPA-BI dose groups for maternal, ovarian or uterine data. There were no treatment-related effects on any fetal parameters from BPA-BI treatment. Based on the results of this study, BPA-BI is not a developmental toxin.

Reference: Morseth, S. L. (1987) Unpublished report number HLA 349-265 entitled "Rat Teratology Study with BPA-DA, BPA-BI and NMP" dated March 5, 1987 for General Electric Company, Plastics Business Operations, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and
Burdock, G. A. (1985) Unpublished report number 349-326 entitled "Pilot Rat Teratology Study: NMP, BPA-BI, BPA-DA, and a Positive Control" dated October 29, 1985 for General Electric Company, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.

Reliability: (Klimisch Code 1) Reliable without restrictions.